IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re application of:		
Misaki Ishida et al.		Art Unit: 1617
Serial No:	09/837,129	Examiner: Willis, Michael A
Filed:	April 18, 2001	

For: Cosmetic composition

DECLARATION UNDER 37 C.F.R. 1.132

Assistant Commissioner for Patents
Washington, D.C. 20231

Sir:

I, Shinji Hayashi, declare and say as follows:

I am a researcher employed at NOF CORPORATIOIN, the assignee of the above-identified patent application. I studied biological applied chemistry at the Department of Agriculture and Forestry of the Second Cluster of Colleges in University of Tsukuba, and received a bachelor's degree in Agriculture in 1991. Since April 1991, I have been employed by NOF CORPORATIOIN and I have been involved in the study of percutaneous drug absorption and the research for materials that can be used as active ingredients in cosmetics.

I am one of the inventors of the invention described in the above-identified patent application, and I am therefore fully familiar with the subject matter of the invention.

The following experiments are given in order to demonstrate that each of an extract obtained from Citrus tachibana provides an excellent whitening effect, but the orange extract used in Dornoff does not provide a whitening effect.

Experiments

Evaluation of whitening effect: suppression of melanin production

According to the process described in Production Example 1 of the present specification, a kippi extract was obtained from dried peel of Citrus tachibana. In more detail, the extract was obtained by pulverized dried peel of Citrus tachibana for 5 days in 95% ethanol in an amount so 5 times the weight of the dried peel, followed by filtration and concentration as described on page 14, lines 15 to 19 of the specification. Furthermore, from the kippi extract, compounds of formulae (II) to (V) were obtained as polymethoxyflavones according to the process of the Production Example 1.

Compound of formula (II): 3,5,6,7,8,3',4'-heptamethoxyflavone (Mw. 432)

Compound of formula (III): 5,6,7,8,3',4'-hexamethoxyflavone (i.e., nobiletin; Mw.402)

Compound of formula (IV): 5,6,7,8,4'-pentamethoxyflavone (i.e., tangeretin; Mw.372)

Compound of formula (V): 5,7,8,3',4'-pentamethoxyflavone (Mw.372)

Pharcolex Orange (produced by ICHIMAMU PHARCOS CO., LED.) was provided as an orange extract that satisfies the standard of orange extract described in the Japanese Cosmetic Ingredients Codex.

The above-mentioned substances were used as test samples.

Using the test samples, the effect of suppressing melanin production of human melanoma cell (HM3KO) was examined in vitro according to the following method.

First, HM3KO was cultured to about 5×10^5 by a conventional method, collected by centrifugation to obtain pellets, and inoculated the pellet to culture dishes of 10cm diameter containing an Eagle's medium supplemented with 10% fetal bovine serum and cultured at 37 °C for 24 hours. Thereafter, each test sample was added to each of the culture dishes so that the final concentration of polymethoxyflavone was 10μM. In more detail, each of the compounds (II), (III), (IV), and (V) is added to the medium so that the final concentration of each compound was 10μM. In case of the kippi extract , the polymethoxyflavone contents of this extract was measured, and the extract was added so that the total amount of the polymethoxyflavones (i.e., compounds (II), (III), (IV), and (V)) was 10μM. Pharcolex Orange (i.e., orange extract) was added to the medium so that the concentration of Pharcolex Orange was 4%. Swift describes that the concentration of total of nobiletin and other closely related compounds (i.e., polymethoxyflavones) is 0.1g per 1L of juice squeezed from orange peel. According to the description of Swift, the 4% Pharcolex Orange may equal to 10μM of polymethoxyflavones because molecular weights of polymethoxyflavones are about 400 as described in a compound list mentioned above.

Then, all the resultant mixtures were cultivated for 6 days. After the cultivation, cells were collected by centrifugation, and 1ml of 2N sodium hydroxide aqueous solution was added thereto to obtain cell lysate. The absorbance in a wavelength of 410nm of the cell lysate was measured with a spectrophotometer. Here, the absorbance of the cell lysate to which a test sample was not added is defined as 100% of the melanin production, and the amounts of melanin production are shown as relative values, measuring the absorbance of each cell lysate. Table 1 shows the results.

Table 1

Test Sample	Concentration of PMF(s)*a in medium (μ M)	Melanin production (% of control)
Control	0	100
Kippi extract	10	55
3,5,6,7,8,3',4'-Heptamethoxyflavone : formula (II)	10	58
5,6,7,8,3',4'-Hexamethoxyflavone : formula (III)	10	42
5,6,7,8,4'-Pentamethoxyflavone : formula (IV)	10	61
5,7,8,3',4'-Pentamethoxyflavone : formula (V)	10	67
Pharcolex Orange	10*b	180

*a: Polymethoxyflavone(s)

*b: an estimate deduced from the description of Swift

As seen from Table 1, each of the test samples of the kippi extract and compounds of formulae (II) to (V) effectively suppressed melanin production of HM3KO. On the contrary, the test sample of Pharcolex Orange does not have such an effect. Furthermore, it can be understood that the Pharcolex Orange disadvantageously affect the suppression of melanin production. The amount of melamine production is higher than that of the control.

Results:

The foregoing experimental results show that the cosmetic composition comprising an extract obtained from Citrus tachibana and the composition comprising a polymethoxyflavone obtained from the extract, both containing polymethoxyflavone(s) in an amount that is in the scope of the present invention have an excellent whitening effect. On the contrary,

the composition of Dornoff comprising an orange extract does not have such an effect. Furthermore, the melanin production value was 180%, and thus, it was demonstrated that the composition of Dornoff disadvantageously affect the whitening effect.

I, Shinji Hayashi, hereby declare that all the statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements were made with knowledge that willful false statements and the like are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code, and that such willful false statements may jeopardize the validity of the application or any patent issued therein.

Shinji Hayashi

Shinji Hayashi

May. 30. 2003

Date